CLAIMS

1. A method for determining HIV-1 subtypes, characterized by comprising the steps of amplifying nucleic acid using as a target sequence a portion of a nucleotide sequence of the env gene of HIV-1, where at least one of the 5′ terminal and 3′ terminal nucleotide sequences is different depending on the HIV-1 subtype, and detecting the subtype depending on whether or not the nucleic acid has been amplified.

2. The method according to Claim 1, wherein the target sequence is 100 to 2500 nucleotides long.

3. The method according to Claim 1, wherein the sequence from the 1^{st} through 30^{th} bases from the 3′ terminal and/or 5′ terminal of the target sequence is different depending on the subtype.

4. The method according to Claim 3, wherein the 3′ terminal of the target sequence is in the C3 region of the env gene of HIV-1.

5. The method according to Claim 4, wherein the 5′ terminal of the target sequence is in the C2 region of the env gene of HIV-1.

6. The method according to Claim 1, wherein different amplification reactions are carried out using different pairs of primers, and different subtypes are detected.

7. The method according to Claim 6, wherein at least two different subtypes are detected by carrying out amplification at least twice with different pairs of primers using primer pairs consisting of a primer (primer 1) that includes a sequence complementary to a portion of the nucleotide sequence (nucleotide sequence 1) that differs depending on subtype in the C3 region of the env gene of HIV-1, and a primer (primer 2) that includes a sequence complementary to a portion of the nucleotide sequence (nucleotide sequence 2) of the C2 region of the env gene of HIV-1.

8. The method according to Claim 1, wherein a first amplification reaction is carried out with a first pair of primers using as a target sequence a portion of a nucleotide sequence of the env gene of HIV-1, a second amplification reaction is then carried out with a second pair of primers using as a target sequence a portion of said nucleotide sequence, where at least one of the 5' terminal and 3' terminal nucleotide sequences is different depending on the HIV-1 subtype, and the subtype is detected depending on whether or not the nucleic acid has been amplified by the second amplification reaction.

9. The method according to Claim 8, wherein the second pair of primers consists of a primer (primer 1) that includes a sequence complementary to a portion of the

nucleotide sequence (nucleotide sequence 1) that differs depending on subtype in the C3 region of the env gene of HIV-1, and a primer (primer 2) that includes a sequence complementary to a portion of the nucleotide sequence (nucleotide sequence 2) of the C2 region of the env gene of HIV-1; and the first pair of primers consists of a primer (primer 3) that includes a sequence complementary to a portion of a nucleotide sequence (nucleotide sequence 3) of a region downstream of the 3' terminal of nucleotide sequence 1 of the env gene of HIV-1, and a primer (primer 4) that includes a sequence complementary to a portion of a nucleotide sequence (nucleotide sequence 4) of a region upstream of the 5' terminal of nucleotide sequence 2 of the env gene of HIV-1.

10. The method according to Claim 8, wherein at least two subtypes are distinguished by repeating at least once, with different pairs of second primers, a series of operations comprising: a first amplification reaction that is carried out with the first pair of primers using as a target sequence a portion of a nucleotide sequence of the env gene of HIV-1; a second amplification reaction that is then carried out with the second pair of primers using as a target sequence a nucleotide sequence within said target sequence; and the detection of subtypes depending on whether or not the nucleic acid has been amplified by the

second amplification reaction.

11. The method according to Claim 10, wherein subtypes A, B, C, and E are distinguished by:

(a) detecting subtype A using as the first primer pair a mixture of primer 12A containing nucleotide sequence GCAATAGAAAAATTCTCCTC (Sequence ID No. 5) and primer 12B containing nucleotide sequence ACAGTAGAAAAATTCCCCTC (Sequence ID No. 6), and a mixture of primer 9AE containing nucleotide sequence CACAGTACAATGCACACATG (Sequence ID No. 8) and primer 9B containing nucleotide sequence CACAGTACAATGTACACATG (Sequence ID No. 9), and using as the second primer pair primer 11QA1 containing nucleotide sequence CTCCTGAGGAGTTAGCAAAG (Sequence ID No. 27) and primer 10U containing nucleotide sequence CTGTTAAATGGCAGTCTAGC (Sequence ID No. 20);

(b) detecting subtype B using as the first primer pair a mixture of primer 12A containing nucleotide sequence GCAATAGAAAAATTCTCCTC (Sequence ID No. 5) and primer 12B containing nucleotide sequence ACAGTAGAAAAATTCCCCTC (Sequence ID No. 6), and a mixture of primer 9AE containing nucleotide sequence CACAGTACAATGCACACATG (Sequence ID No. 8) and primer 9B containing nucleotide sequence CACAGTACAATGTACACATG (Sequence ID No. 9), and using as the second primer pair

primer 11VB containing nucleotide sequence CACAATTAAAACTGTGCATTAC (Sequence ID No. 28) and primer 10U containing nucleotide sequence CTGTTAAATGGCAGTCTAGC (Sequence ID No. 20);

(c) detecting subtype C using as the first primer pair a mixture of primer 12A containing nucleotide sequence GCAATAGAAAAATTCTCCTC (Sequence ID No. 5) and primer 12B containing nucleotide sequence ACAGTAGAAAAATTCCCCTC (Sequence ID No. 6), and a mixture of primer 9AE containing nucleotide sequence CACAGTACAATGCACACATG (Sequence ID No. 8) and primer 9B containing nucleotide sequence CACAGTACAATGTACACATG (Sequence ID No. 9), and using as the second primer pair primer 11XC containing nucleotide sequence TTGTTTTATTAGGGAAGTGTTC (Sequence ID No. 29) and primer 10UC containing nucleotide sequence CTGTTAAATGGTAGTCTAGC (Sequence ID No. 24); and

(d) detecting subtype E using as the first primer pair a mixture of primer 12A containing nucleotide sequence GCAATAGAAAAATTCTCCTC (Sequence ID No. 5) and primer 12B containing nucleotide sequence ACAGTAGAAAAATTCCCCTC (Sequence ID No. 6), and a mixture of primer 9AE containing nucleotide sequence CACAGTACAATGCACACATG (Sequence ID No. 8) and primer 9B containing nucleotide sequence CACAGTACAATGTACACATG

(Sequence ID No. 9), and using as the second primer pair primer 11WE containing nucleotide sequence CTCTACAATTAAAATGATGCATTG (Sequence ID No. 30) and primer 10U containing nucleotide sequence CTGTTAAATGGCAGTCTAGC (Sequence ID No. 20).

12. The method according to Claim 8, wherein at least two subtypes are distinguished by repeating at least once, with different pairs of first and second primers, a series of operations comprising: a first amplification reaction that is carried out with a first pair of primers using as a target sequence a portion of a nucleotide sequence of the env gene of HIV-1; a second amplification reaction that is then carried out with a second pair of primers using as a target sequence a nucleotide sequence within said target sequence; and the detection of subtypes depending on whether or not the nucleic acid has been amplified by the second amplification reaction.

13. The method according to Claim 12, wherein subtypes A, B, and E are distinguished by:

(a) detecting subtype A using as the first primer pair primer 12A containing nucleotide sequence GCAATAGAAAAATTCTCCTC (Sequence ID No. 5) and primer 9AE containing nucleotide sequence CACAGTACAATGCACACATG (Sequence ID No. 8), and using as the second primer pair primer 11QA containing nucleotide sequence

CTCCTGAGGGGTTAGCAAAG (Sequence ID No. 1) and primer 10 containing nucleotide sequence AAATGGCAGTCTAGCAGAAG (Sequence ID No. 4);

(b) detecting subtype B using as the first primer pair primer 12B containing nucleotide sequence ACAGTAGAAAAATTCCCCTC (Sequence ID No. 6) and primer 9B containing nucleotide sequence CACAGTACAATGTACACATG (Sequence ID No. 9), and using as the second primer pair primer 11BB containing nucleotide sequence CTGTGCATTACAATTTCTGG (Sequence ID No. 2) and primer 10 containing nucleotide sequence AAATGGCAGTCTAGCAGAAG (Sequence ID No. 4); and

(c) detecting subtype E using as the first primer pair primer 12E containing nucleotide sequence GCAATAGAAAAATTCCCCTC (Sequence ID No. 7) and primer 9AE containing nucleotide sequence CACAGTACAATGCACACATG (Sequence ID No. 8), and using as the second primer pair primer 11QE containing nucleotide sequence CTCCTGAGGGTGGTTGAAAG (Sequence ID No. 3) and primer 10 containing nucleotide sequence AAATGGCAGTCTAGCAGAAG (Sequence ID No. 4).

14. The method according to Claim 1, further comprising the steps of amplifying nucleic acid using as a target sequence a portion of a nucleotide sequence of the HIV-1 genome, the nucleotide sequence being highly

conserved among all subtypes, and ascertaining the presence or absence of HIV-1 depending on whether or not the nucleic acid has been amplified.

15. The method according to Claim 14, wherein the step for ascertaining the presence or absence of HIV-1 comprises amplifying the nucleic acid with a first primer pair using as a target sequence a portion of a nucleotide sequence of the HIV-1 genome, the nucleotide sequence being highly conserved among all subtypes, then carrying out a second amplifying reaction with a second primer pair using as a target sequence a nucleotide sequence in said target sequence, and ascertaining the presence or absence of HIV-1 depending on whether or not the nucleic acid has been amplified.

16. The method according to Claim 15, wherein the primers that are used comprise a mixture of a plurality of upstream primers with differing nucleotide sequences and a plurality of downstream primers with differing nucleotide sequences.

17. The method according to Claim 16, wherein the first primers comprise a mixture of primer 12A containing nucleotide sequence GCAATAGAAAAATTCTCCTC (Sequence ID No. 5), primer 12B containing nucleotide sequence ACAGTAGAAAAATTCCCCTC (Sequence ID No. 6), primer 9AE containing nucleotide sequence CACAGTACAATGCACACATG

(Sequence ID No. 8), and primer 9B nucleotide sequence CACAGTACAATGTACACATG (Sequence ID No. 9), and the second primer pair comprises primer 11LB containing nucleotide sequence AATTTCTGGGTCCCCTCCTG (Sequence ID No. 18), primer 11LAE containing nucleotide sequence AATTTCTAGATCCCCTCCTG (Sequence ID No. 25), primer 11LC containing nucleotide sequence AATTTCTAGGTCCCCTCCTG (Sequence ID No. 26), and primer 10U containing nucleotide sequence CTGTTAAATGGCAGTCTAGC (Sequence ID No. 20).

18. A kit for determining HIV-1 subtypes, comprising primer pairs in which a target sequence is a portion of a nucleotide sequence of the env gene of HIV-1, where at least one of the 5' terminal and 3' terminal nucleotide sequences is different depending on the subtype.